RESEARCH ARTICLE

Vegetation changes at oligotrophic grasslands managed for a declining butterfly

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Abstract

A selection of sites occupied by the EU-protected marsh fritillary (*Euphydryas aurinia*) in western Czech Republic were subjected to a vegetation survey 15 years ago and again recently. In the 66 time-replicated 25 m² plots from 12 sites, representing the diversity of *E. aurinia*-occupied oligotrophic grasslands in the Slavkovský les Protected Landscape Area (and covering a fifth of the currently-occupied Czech sites), we recorded quantitative representation of vascular plants and mosses. We analysed the data using multivariate ordinations, asking how the vegetation changed between the surveys, how was it affected by the conservation management applied and how it affected occupancy by the butterfly larval nests; the vegetation patterns were interpreted using Ellenberg's plant indicator values. Between the two surveys, the overall representation of the larval host plant, *Succisa pratensis*, did not change; tree and herbs layers (both grasses and forbs) increased and the moss layer decreased. Across surveys, the main driver of vascular plants' species composition was moisture, followed by soil reaction and nitrogen, whereas in mosses, nitrogen was the main factor. The main change between the surveys was the increase of nitrogen accompanied by decreased light, probably due to increase of competitively strong plants. Butterfly occupancy declined at sites with high soil moisture and increased at sites with higher soil reaction. Mowing of moist nitrogen-rich sites, but not drier nitrogen-poor sites, increased occupancy, illustrating the need for context-dependent interventions. All the evidence thus shows that *E. aurinia* prefers drier, warmer and less acidic conditions within the generally moist acidic grasslands and that ongoing eutrophication represents a potential problem in the future.

Keywords

Introduction

Due to fluctuating numbers of individuals within populations, short individual life spans, vulnerability to stochastic mortality factors and close specialisation to highly exacting resources (e.g. Dennis (2010)), conservation of declining insects typically relies on conserving their habitats (Settele et al. 2009; van Swaay et al. 2012; Warren et al. 2021). This may require active management interventions, if persistence of a habitat depends on a specific disturbance regime, historically supplied by now outdated land use patterns (Öckinger et al. 2006a, b; Bonari et al. 2017; Sienkiewicz-Paderewska et al. 2020). The latter is often the case of the "semi-natural grasslands" of Europe, land-cover types derived from early-Holocene and perhaps even older vegetation forms (Thomas 1993; Feurdean et al. 2018), hosting an outstanding diversity of endangered species (van Swaay et al. 2006), but suffering a continent-wide decline due to land-use changes (Nilsson et al. 2013) and so increasingly dependent on targeted conservation management.

The marsh fritillary, *Euphydryas aurinia* (Rottenburg, 1775) (Nymphalidae, Nymphalinae) is a polymorphic butterfly, distributed across the Palaearctic temperate zones (Junker et al. 2015; Korb et al. 2016). Within its wide range, it occupies various habitats and displays regional specialisations on various larval host plants (Singer et al. 2002; Liu et al. 2006; Junker and Schmitt 2010; Junker et al. 2010; Meister et al. 2015). Its distribution has declined seriously in Western and Central Europe, where it is restricted to oligotrophic conditions and develops on *Gentiana asclepiadea* L. (Anthes et al. 2003), *Knautia* spp. (Anthes and Nunner 2006), *Scabiosa* spp. (Anthes and Nunner 2006; Scherer and Fartmann 2022) or *Succisa pratensis* Moench (Wahlberg et al. 2002; Konvicka et al. 2003; Schtickzelle et al. 2005). The butterfly is listed in the EU Habitats Directive and its regional persistence increasingly depends on active vegetation management (e.g. Bulman et al. (2007), Scherer and Fartmann (2022)), including reintroductions (Davis et al. 2021).

In the Czech Republic, *E. aurinia* occurs at oligotrophic submontane grasslands in the westernmost part of the country (Hula et al. 2004; Zimmermann et al. 2011a). It is currently restricted to 19 grid squares (square area ≈ 100 km^2; 2.81% of the country's total) and is nationally endangered (Hejda et al. 2017). Within the occupied area (Fig. 1), it is restricted to ≈ 100 separate oligotrophic moist meadow patches, where it displays a classic (*sensu* Hanski 1999) extinction-recolonisation metapopulation dynamic, facilitated by inter-patch movements (Zimmermann et al. 2011b; Junker et al. 2021). Two decades ago, Konvička et al. (2003) described larval preference for prominent *Succisa pratensis* plants growing amidst shorter and nutrient-poor grassland vegetation, a general pattern in Central and Northern Europe (Anthes et al. 2003; Schtickzelle et al. 2005; Betzholtz et al. 2007; Janovský et al. 2016; Pielech et al. 2017; Pschera and Warren 2018).

The currently occupied grasslands were historically utilised as non-intensive pastures and litter meadows. A relatively large area of such grasslands (≈ 5% of the landscape; Junker et al. (2021)) was regionally spared the twin threats of woody encroachment and intensification owing to the existence of a military range, sanitation zones
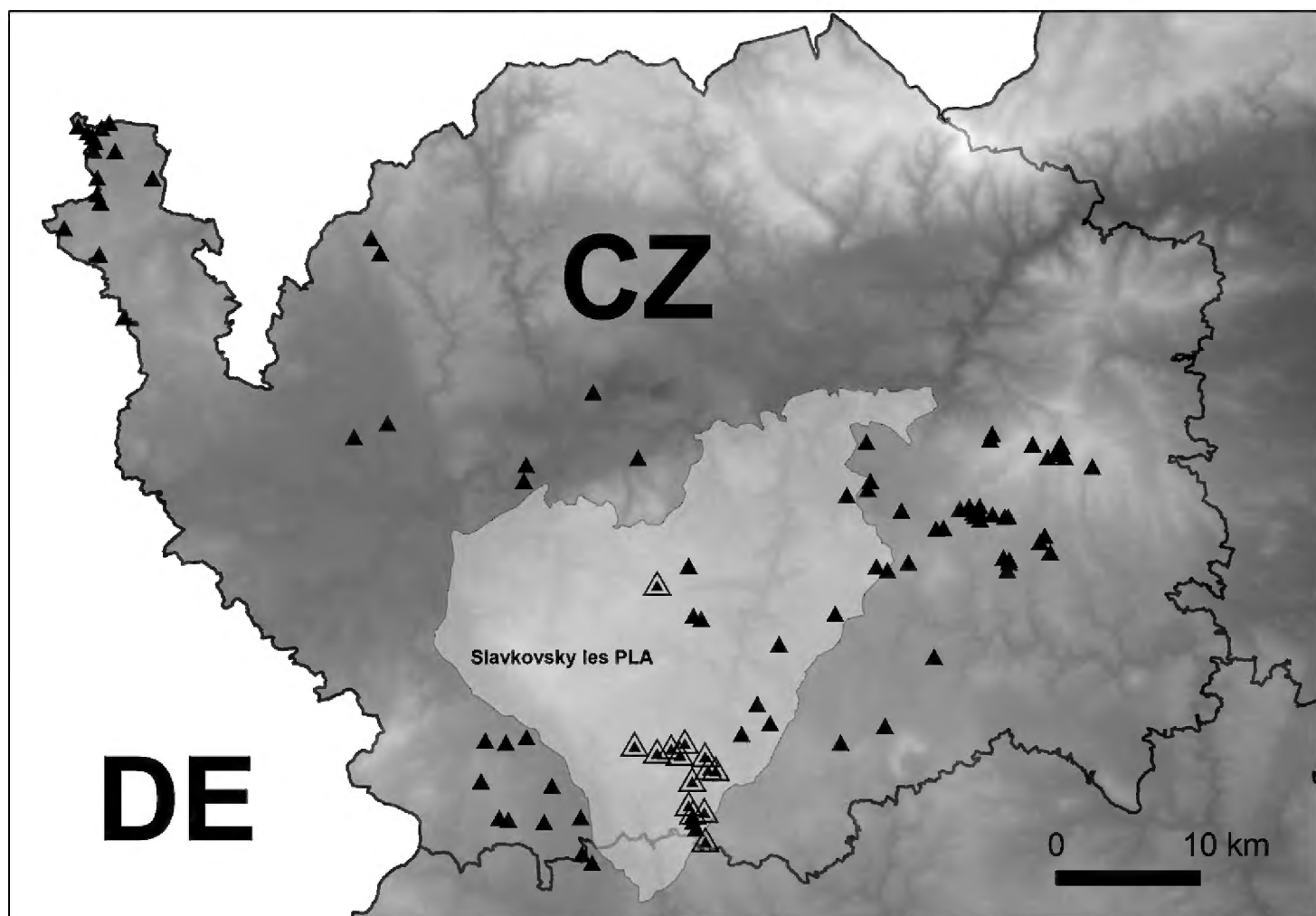
Figure 1. Map of western Czech Republic with the extend of Slavkovský les Protected Landscape Area (green shading), all recently (= after 2000) known colonies of *Euphydryas aurinia* butterfly (black triangles) and colonies where the repeated vegetation recording took place (doubled triangles).

of two freshwater reservoirs and numerous mineral water zones with low agrochemical use. Following the Czech Republic's entry to the EU (2004), selected *E. aurinia* localities became Sites of (European) Community Importance (SCI), purposefully managed for the species. The prevailing management is light machine-mowing with temporary retention of unmown strips and host plant patches. Conservation grazing, applied for *E. aurinia*, for example, in Britain and Sweden (Smee et al. 2011; Johansson et al. 2020), is rarely used, because local beef farms operate with too large herds in relatively intensive regimes. In the wider environs, changes during the last two decades involved re-seeding former arable fields by grass mixtures, concurrent with a shift from wheat or dairy farming to beef ranching.

The targeted management of valuable sites, combined with non-intensive use of the surrounding landscape, should spare the submontane grasslands from further degradation. However, these efforts may not buffer the grasslands from multiple deteriorating factors, operating at both large and local scales. The large-scale threats include eutrophication by increased nitrogen and phosphorus inputs from the atmosphere (Stevens et al. 2010; Roth et al. 2021), known to impair the species' composition of oligotrophic grasslands (Bollens et al. 2001) and climatic effects, manifested by periods of drought and rising temperatures (Filz et al. 2013). The years 2015–2018 were the driest in recorded history in Central Europe (Buras et al. 2020; John et al. 2020)

and, while the average temperatures rose by 1.1 °C compared to pre-industrial situation globally (World Meteorological Organization 2019), they regionally increased by 2.1 °C compared to mid-20th century (Czech Hydrometeorological Institute 2022). The small-scale threats stem from the fact that currently practised interventions differ from those that maintained the biotopes in the past (Valkó et al. 2018; Almásy et al. 2021; Kuhn et al. 2021). Light machine-mowing effects differ from those of manual mowing or low intensity grazing. For instance, mowing may support grasses at the expense of forbs (Stammel et al. 2003; Mládková et al. 2015) and homogenises vegetation due to the absence of small-scale sod disturbance (Tälle et al. 2016).

In this paper, we compare vegetation changes at *E. aurinia* colonies over a 15-year period and relate them to vegetation management of the sites and occupancy patterns by the butterfly. We analyse vegetation records from a selection of the occupied sites in 2005–7, i.e. in the time when a conservation regime was established and replication of the records from identical spots in 2020–21. In the intervening time, the sites were annually monitored for the presence of *E. aurinia* larval nests. Interpreting the changes in the plant species composition using readily available information on individual plants' ecological requirements (i.e. their indicator values: Ellenberg et al. (1992)) provides insights into drivers of the plant community change. Specifically, we asked: (1) How did the vegetation change between the two surveys? (2) Was the vegetation change linked to the management of the sites? (3) Were the vegetation changes linked to utilising individual sites by the butterfly? Further, we test the specific hypotheses: (4) that the interventions applied changed the vegetation composition (4a), that occupancy of the sites by *E. aurinia* (4b) and abundance of its larval nests (4c) changed between the two surveys and that management changed occupancy (4d) and nest counts (4e).

Materials and methods

Study system and data collection

Euphydryas aurinia is a univoltine butterfly, with adult generation from late May to late June. *Succisa pratensis*, its locally used host plant, is a late-season richly blooming perennial, forming prostate leaf rosettes in early summer and growing to ≈ 70 cm in August to September. Pre-hibernation larvae form conspicuous silk-woven communal nests on the plants and finish development solitarily in the following spring.

Annual counts of the pre-hibernation larval groups at all known *E. aurinia* sites, i.e. habitat patches of varying size occupied by the butterfly and utilised for its larval development began in 2001. This was combined with searching for hitherto unknown sites in the wider environs of the occupied ones (Hula et al. 2004; Junker et al. 2021), in connection with vegetation mapping by the Czech Nature Conservation Agency (Härtel et al. 2009). The number of known sites gradually increased from 17 in 2001 to 97 in 2022. Their summed area is 295 ha (mean area 3.0 ± 7.47, range 0.08–70). Following discovery of a site, it was visited annually in September for larval nest counts and overall assessment.

The Central European vegetation classification system (Chytrý et al. 2007, 2011) classifies the occupied grasslands as intermittently wet *Molinia* meadows (association *Junco effusi-Molinietum caeruleae* Tüxen, 1954), fens (alliance *Caricion canescenti-nigrae* Nordhagen, 1937, association *Caricetum nigrae* Braun, 1915), wet meadows (alliance *Calthion*, association *Angelico sylvestris-Cirsietum palustris* Darimont ex Balátová-Tuláčková, 1973) and short grasslands with *Nardus stricta* alliance *Violion caninae* (association *Festuca capillatae-Nardetum strictae* Klika & Šmarda, 1944). In addition to *E. aurinia*, they host multiple regionally declining plants and animal taxa (Tájek 2006, 2007, 2010, 2014).

Approximately one quarter (27) of the 97 *E. aurinia* sites are situated within Slavkovský les Protected Landscape Area (PLA), volcanic mountains (maximum altitude: Lesný 983 m, *E. aurinia* colonies at 520–810 m), where the oligotrophic grasslands are present on the elevated plateaux. The PLA administration oversees the management of the sites within its border. From twelve such sites (mean area 5.4 ± 6.05 SD, range 0.3–18.7 ha), 66 vegetation relevés (mean per site: 5.5 ± 4.64, range 1–18) were recorded in 2005–6 and the recording was replicated in 2020–21. The majority (51) of the relevés were selected using the randomisation process in ArcView 3.2, bounded by boundaries of known *E. aurinia* sites. The additional fifteen relevés from the same twelve sites were positioned arbitrarily, while documenting the vegetation of the then established SCIs. During the 2005–6 survey, all the relevés were photographed, georeferenced and marked in the field by iron nails. During the 2020–21 survey, they were relocated using the GPS points and a Minelab X-TERRA 505 metal detector and marked with painted wooden poles. The relocating was done prior to the vegetation season (April) to avoid trampling the vegetation.

Recording the vegetation followed a standard procedure for grasslands in Central Europe (Braun-Blanquet 1964): the area of each relevé was 25 m² and within it, covers of all vascular plant species present, plus covers of all mosses, all of which were recorded using the 9-point scale: 1: up to 3 individuals, 2: < 1% cover; 3: 1–5%, 4: ≈ 5%, 5: 5–15%; 6: 15–25%; 7: 25–50%; 8: 50–75%; 9: 75–100%. The records were taken separately for moss (E_0), herb (E_1), shrub (E_2) and tree (E_3) vertical layers and total covers of the layers per relevé (E_2 and E_3 as the ground projection to the relevé surface) were recorded. In 2020–21, recording followed the identical procedure.

We tabulated the vascular plants' indicator values for light (L), moisture (H), nitrogen (N), soil reaction (R) and temperature (T) using Ellenberg et al. (1992) and mosses' values using Düll (2001) and Simmel et al. (2021).

Statistical analyses

As all the analyses are based on past-present comparisons from identical relevés; we used Wilcoxon-matched pairs tests to compare species richness, covers of vegetation layers, the representation of the host plant *S. pratensis* and covers of grasses and forbs, between the present and past surveys.

Changes in the species' composition of vegetation relevés were analysed using multivariate ordination techniques, always separately for vascular plants, mosses and all plants, in CANOCO for Windows 5.00 (Ter Braak and Smilauer 2012). Given the rather long gradients in the samples' species composition (all plants: 3.5, vascular plants: 3.6, mosses: 4.2), we used unimodal methods and, due to the horseshoe effects apparent in unconstrained ordination biplots, we used detrended correspondence analysis (DCA) for unconstrained and detrended canonical correspondence analysis (DCCA) for constrained calculations, always detrending by segments. DCA ordinates the species according to their distribution in samples. DCCA constrains the ordination by the predictor(s) of interest, testing the significance of species composition ~ predictors relationships via a Monte-Carlo test (999 permutations).

'Survey' (past vs. present, two states) was one of the explanatory variables used to compare plants species composition. Further explanatory variables were: 'Management', factorially coded as the intervention applied during the three years centred by the year of survey for the section of the site containing the relevé; we distinguished Neglect (past/present: 59/25 relevés), Conventional mowing (standard farm machinery, cutting the entire locality at once) (6/1) and Conservation mowing (light machinery, proceeding by strips or a chequer, intentionally leaving aside some *S. pratensis* patches) (1/40). 'Nests count' denotes the mean annual number of nests per site in the years 2005–9 (past) and 2017–21 (present). Finally, 'Occupancy' (two states, 1/0) denotes whether a ≈ 20 m diameter circle centred in the centre of the relevé contained *E. aurinia* larval nests(s) in the year of vegetation survey; this was assessed during the first week of September following the surveys.

As the numbers of relevés were unbalanced amongst sites and the sites varied, for example, in altitude, soil and moisture conditions, we relied on covariables-controlled partial ordinations to filter out the background variation amongst sites. We used two options. First, we defined a geography model explaining the plant species composition of the relevés by a linear combination of latitude, longitude and altitude; and their 2nd-order polynomials and interactions, defining the most appropriate covariate model using the CANOCO forward selection procedure. Second, we entered site identities as a 12-state factor.

In the DCCAs, we reflected the time-replicated sampling by a split-plot permutation design, in which the two temporal replications per relevé represented whole plots, which were permuted as time series and the 66 relevés represented split plots, permuted randomly.

To interpret the ordination results by Ellenberg's bioindication values, we followed the fourth-corner approach (Legendre et al. 1997; Dray and Legendre 2008), which relates two tables, that of species composition of samples and that of relevant predictors, to a table of constituent species' traits. Specifically, we related the ordination axes returned by DCAs/DCCAs to a table of Ellenberg's indicator values using redundancy analysis (RDA), an ordination method analogous to linear regression. We selected significant traits via forward selection procedures, again with 999 permutations.

Testing the specific hypotheses 4a – 4e relied on testing interactions amongst predictors. If predictors *a* and *b*, for example, 'survey' and 'management', both influence

the species composition y, their effect can be additive, $y \sim a + b$, or multiplicative, $y \sim a + b + a*b$, the latter implying that 'management' exerted different effects during the first and second 'survey'. Then, setting the linear terms as covariates, $y \sim a*b \mid a+b$ tests for the separate effect of the interaction, i.e. whether 'management' affected the vegetation differently during the first and second 'survey'. In cases when the interaction terms were significant, we again interpreted the results using plants' indicator values.

Results

In the past, the 66 relevés contained 167 species of vascular plants ($\bar{X} \pm$ SD/median per relevé: $37.8 \pm 9.53/39.5$), one in E_3 and two in E_2 layers and 63 species of mosses ($5.4 \pm 2.89/5$) (Suppl. material 1). At present, there were 178 species of vascular plants ($42.4 \pm 9.22/41.5$), three in E_3 and five in E_2 layers and 50 species of mosses ($5.7 \pm 2.49/5.5$). Per relevé, number of vascular plants' species was significantly higher at present (T = 190.5, W = 5.60, p << 0.001), whereas the number of moss species did not change significantly (T = 670.5, W = 1.62, p = 0.11).

The butterfly's host plant, *Succisa pratensis*, was represented in 63/59 relevés in the past/at present. Neither its presence/absence within the relevés (sign test: Z = 1.5, p = 0.13) nor its percentage covers within the relevés (past: $3.4 \pm 1.72/3$; present $3.2 \pm 1.72/3$; T = 332.0, W = 1.28, p = 0.20) changed between the surveys. Regarding the percentage covers of vegetation layers, E_3 increased from the past (0.2 ± 0.85 SD/ 0) to the present (2.2 ± 0.62 SD/0) (T = 2.0, W = 2.90, p < 0.01); E_2 did not change (past: 0.7 ± 2.59 SD/0; present: 0.9 ± 3.30 SD/0; T = 62.5, W = 0.28, p = 0.78); E_1 increased at the present (past: $75.0 \pm 11.51/76.5$; present: $82.3 \pm 10.14/85$; T = 160.5, W = 5.01, p << 0.001); and E_0 decreased (past: $28.5 \pm 29.89/12$; present: 18.1 ± 22.09 SD/10; T = 401.5, W = 3.52, P < 0.001). Splitting E_1 into forbs and grasses (the latter including Poaceae, Cyperaceae and Junceae) showed that both groups increased their cover representation (forbs: past $54 \pm 13.3/55$, present $60 \pm 14.4/61$; T = 309.5, W = 4.57, P << 0.001; grasses: past $40 \pm 9.4/42$, present $45 \pm 8.8/45$, T = 364.0, W = 4.52, P << 0.001).

In the partial indirect DCA ordinations of species composition of samples, site always fitted more variation than geography (Table 1). We, therefore, used site as the covariate in all subsequent analyses. In the indirect DCA analyses for vascular plants (Fig. 2a, c), the primary gradients differentiated samples from wet waterlogged sites, with vascular plants, such as *Filipendula ulmaria*, *Equisetum fluviatile*, *Potentilla palustre* or *Viola palustris*, from drier vegetation containing, for example, *Agrostis capillaris*, *Nardus stricta* or *Hypericum maculatum*. Secondary gradients run from tall-growing, nutrients-demanding vascular plants, such as *Descampsia caespitosa*, *Poa pratensis*, *Cirsium heterophylum*, towards short-growing species characteristic for nutrient-poor or frequently disturbed substrates, such as *Potentilla erecta*, *Valeriana dioica*, *Carex nigra*. *Succisa pratensis*, the host plant of *E. aurinia*, was located within the latter group. For mosses (Fig. 2b, d), the primary gradient differentiated species requiring low nutrient sites, such as *Sphagnum warnstorfii*, *S. teres*, *Tomentypnum nitens*, from

species tolerating high nutrients, such as *Hygroamblystegium humile* or *Brachythecium mildeanum*. The secondary gradient differentiated hygrophilous (e.g. *Hygroamblystegium humile*) from relatively xerophilous (e.g. *Pleurozium schreiberi*) species. Interpreting the indirect ordinations by indicator values (Table 1) corroborated that in vascular plants, the primary gradients were moisture-driven and secondary gradients nutrients-, light- or soil reaction-driven. This differed from mosses, in which nutrients drove the primary gradient. See Suppl. material 1 for ordination scores of all species.

Constraining the ordinations by the predictors of interest returned consistent results for vascular plants, mosses and all plants (Table 2). The amounts of variation attributable to the predictors were always low, but the composition of relevés was significantly related to the predictors even when controlled for site identity, except for 'occupancy' and 'nest count' in the case of mosses. Whereas in vascular plants and all plants, results of almost all partial DCCAs were attributable to the plants' indicator values, only the partial ordination constrained by 'survey' was explicable by indicator values for mosses.

'Survey' exerted significant effects for both vascular plants and mosses, revealing that vegetation changed from past to present consistently across the sites. Interpretation
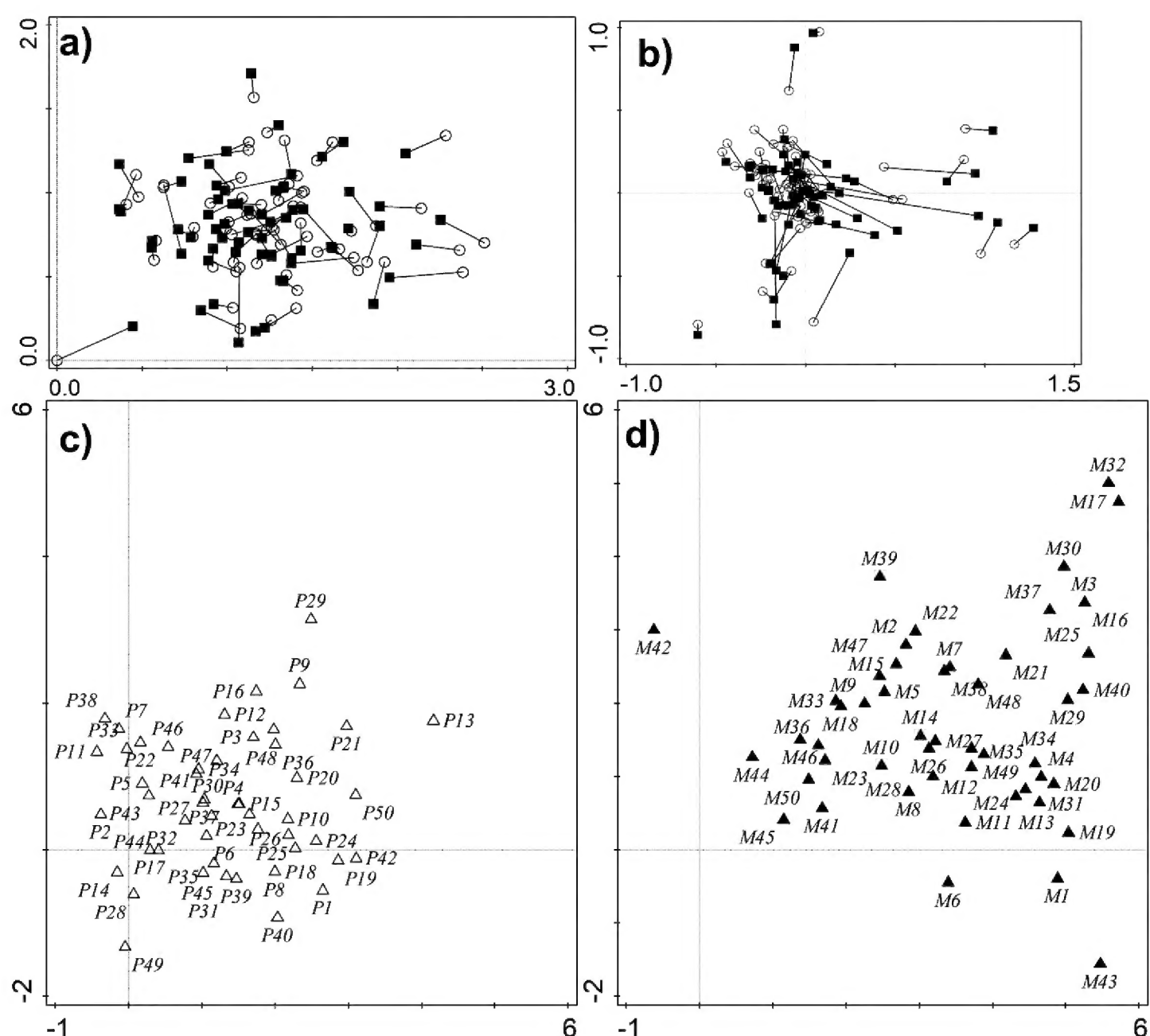
by indicator values (Table 2, Fig. 3a, e) showed lower values for nitrogen (vascular plants, mosses, all plants) and higher values for light (vascular plants and all plants) in the past. Inspection of the ordination diagrams revealed a higher representation of vascular plants, such as *Dactylorhiza majalis*, *Achillea ptarmica* or *Festuca ovina* and mosses, such as *Plagiomnium cuspidatum* or *Thuidium tamariscunum* in the past and a higher representation of vascular plants, such as *Cirsium arvense* or *Festuca pratense* and mosses, such as *Drepanocladus aduncus* or *Polytrichum formosum* at present. *Succisa pratensis* inclined towards the past, likely due to intervening loss from some relevés.

In the ordinations for 'management', primary gradients differentiated conservation and conventional mowing from neglect, whereas secondary gradients differentiated conventional and conservation mowing. In both vascular plants and all plants ordinations, *Succisa pratensis* ended up near the centres of ordination spaces. Interpretation of the gradients, significant only for vascular and all plants, showed an association of low indicator values for nitrogen and high indicator values for moisture, with neglect. Contrarily, conservation mowing was associated with higher indicator values for temperature and nitrogen (Fig. 3b).

Figure 2. DCA ordination biplots ordinating samples (**a, b**) and species (**c, d**) recorded in relevés taken from sites occupied by the *Euphydryas aurinia* butterfly, on the first and second ordination axes after controlling for site identity. See Table 1 for ordination results. In the plots **a** and **b**, past sampling is denoted by open circles, present sampling by black squares; the lines connecting past and present samples can be viewed as vectors of vegetation change. Plots **c** and **d** each present 50 best-fitting species. Vascular plants: P1 – *Agrostis canina*, P2 – *A. capillaris*, P3 – *A. stolonifera*, P4 – *Angelica sylvestris*, P5 – *Anthoxanthum odoratum*, P6 – *Bistorta major*, P7 – *Briza media*, P8 – *Cardamine pratensis*, P9 – *Carex echinate*, P10 – *C. nigra*, P11 – *C. pallescens*, P12 – *C. panicea*, P13 – *C. rostrata*, P14 – *Cirsium heterophyllum*, P15 – *C. palustre*, P16 – *Crepis paludosa*, P17 – *Deschampsia cespi*tosa, P18 – *Epilobium palustre*, P19 – *Equisetum fluviatile*, P20 – *E. palustre*, P21 – *Eriophorum angustifolium*, P22 – *Festuca ovina*, P23 – *F. rubra*, P24 – *Filipendula ulmaria*, P25 – *Galium palustre*, P26 – *G. uliginosum*, P27 – *Holcus lanatus*, P28 – *Hypericum maculatum*, P29 – *Juncus atriculatus*, P30 – *J. conglomeratus*, P31 – *J. effusus*, P32 – *Lathyrus pratensis*, P33 – *Luzula campestris*, P34 – *L. multiflora*, P35 – *Lychnis flos-cuculi*, P36 – *Mentha arvensis*, P37 – *Myosotis nemorosa*, P38 – *Nardus stricta*, P39 – *Poa pratensis*, P40 – *P. trivialis*, P41 – *Potentilla erecta*, P42 – *P. palustris*, P43 – *Ranunculus acris*, P44 – *R. auricomus*, P45 – *Rumex acetosa*, P46 – *Succisa pratensis*, P47 – *Tephroseris crispa*, P48 – *Valeriana dioica*, P49 – *Veronica chamaedrys*, P50 – *Viola palustris*. Mosses: M1 – *Aulacomnium androgynum*, M2 – *A. palustre*, M3 – *Brachythecium albicans*, M4 – *B. mildeanum*, M5 – *B. rivulare*, M6 – *B. rutabulum*, M7 – *Breidleria pratensis*, M8 – *Bryum pseudotriquetrum s. l.*, M9 – *Calliergonella cuspidate*, M10 – *Campylium stellatum*, M11 – *Cephalozia bicuspidate*, M12 – *Ceratodon purpureus*, M13 – *Cirriphyllum piliferum*, M14 – *Climacium dendroides*, M15 – *Dicranella heteromalla*, M16 – *Dicranum montanum*, M17 – *D. polysetum*, M18 – *D. scoparium*, M19 – *Fissidens osmundoides*, M20 – *Hygroamblystegium humile*, M21 – *Chiloscyphus coadunatus*, M22 – *C. cuspidatus*, M23 – *C. polyanthos*, M24 – *C. profundus*, M25 – *Leptodictyum riparium*, M26 – *Plagiomnium affine*, M27 – *P. ellipticum*, M28 – *P. undulatum*, M29 – *Plagiothecium denticulatum s. l.*, M30 – *Pleurozium schreberi*, M31 – *Polytrichum commune*, M32 – *P. longisetum*, M33 – *Pseudocampylium radicale*, M34 – Rhizomnium punctatum, M35 – *Rhytidiadelphus squarrosus*, M36 – *Sphagnum capillifolium*, M37 – *S. fallax*, M38 – *S. fimbriatum*, M39 – *S. flexuosum*, M40 – *S. girgensohnii*, M41 – *S. magellanicum* agg., M42 – *S. palustre*, M43 – *S. riparium*, M44 – *S. teres*, M45 – *S. warnstorfii*, M46 – *Straminergon stramineum*, M47 – *Thuidium assimile*, M48 – *T. delicatulum*, M49 – *T. recognitum*, M50 – *Tomentypnum nitens*.

Table 1. Results of indirect DCA ordinations of the vegetation composition at plots established within grasslands occupied for the butterfly *Euphydryas aurinia* in western Czech Republic, including partial ordinations controlled for the covariates site and geography and (in bold italic) the RDA interpretation of the DCA ordination axes by the Ellenberg's indicator values of the constituent plant species.

		Ax1	Ax2	Ax3	Ax4	Var%	RDA$_{Ax1}$	RDA$_{all\ axes}$	Relating DCA axes to indicator values
Vascular plants									
~	Eigenvalues	0.193	0.130	0.089	0.057	3.0			
	Var%	10.0	16.8	21.4	24.3				
	Interpretation	*0.092*	*0.049*	*0.036*		*16.4*	*6.4****	*13.7****	*Ax1: +H, Ax2: -R, Ax3: -N*
~ | site	Eigenvalues	0.163	0.087	0.064	0.045	1.4			
	Var%	10.4	16.0	20.1	22.9				
	Interpretation	*0.082*	*0.013*	*0.003*		*8.4*	*5.7****	*6.9****	*Ax1: +H, +T, +R*
~|lat+lat²	Eigenvalues	0.063	0.035	0.032	0.002	2.3			
	Var%	6.8	11.2	14.4	17.2				
	Interpretation	*0.055*	*0.035*	*0.031*	*0.001*	*11.4*	*3.2****	*7.3****	*Ax1: -H, Ax2: +L, Ax3: -N, Ax4: +R*
Mosses									
~	Eigenvalues	0.591	0.455	0.277	0.232	3.3			
	Var%	10.9	19.29	24.39	28.6				
	Interpretation	*0.033*				*1.9*	*1.9⁺*		*Ax1: +H*
~ | site	Eigenvalues	0.389	0.280	0.248	0.185	1.4			
	Var%	8.5	15.1	20.6	24.8				
	Interpretation	*0.051*				*3.7*	*3.6**		*Ax1: -N*
~ | lat+lon+lat²	Eigenvalues	0.460	0.294	0.265	0.196	1.8			
	Var%	9.4	15.5	20.9	24.9				
	Interpretation	*0.051*				*3.7*	*3.7**		*Ax1: -N*
All plants									
~	Eigenvalues	0.219	0.129	0.094	0.070	2.8			
	Var%	9.7	15.5	19.7	22.8				
	Interpretation	*0.083*	*0.075*	*0.026*		*17.1*	*5.8****	*14.4****	*Ax1: +H, Ax2: -N, Ax3: +R*
~ | site	Eigenvalues	0.177	0.129	0.059	0.061	1.4			
	Var%	9.7	16.8	20.5	23.3				
	Interpretation	*0.076*	*0.021*	*0.012*		*9.4*	*5.2****	*7.7****	*Ax1: +H, Ax2: -N, Ax3: +R*
~ | lat+lat²	Eigenvalues	0.195	0.130	0.081	0.055	2.1			
	Var%	9.4	15.6	19.5	22.2				
	Interpretation	*0.098*	*0.040*	*0.020*	*0.003*	*14.4*	*5.2****	*9.1****	*Ax1: +H, Ax2: -N+L, Ax3: +R*

Legend: Ax1-Ax4 refer to ordination axes, Var% to variation explained by the respective ordination axis/model.
Monte-Carlo test results – ⁺: $p < 0.1$, *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$.

The ordinations for 'occupancy', again significant for vascular plants and all plants, showed that presence of *E. aurinia* was associated with low nitrogen and high temperature. *Succisa pratensis* and multiple nitrogen-intolerant species, including Red-listed ones, were associated with occupied sites (e.g. *Parnassia palustris, Carex rostrata, Trientalis europaea, Scorzonera humilis, Dactylorhiza fuchsii, Oxycoccus palustris*). Nitrophilous plants, such as *Urtica dioica, Heracleum sphondylium* or *Plantago major*, were associated with unoccupied conditions (Fig. 3c).

For 'nest count', the explained variation after controlling for site identity was very low, but still indicated that sites with high nest counts were drier and displayed higher soil reaction than those with low nest counts. This was reflected by association of high nest counts with plants, such as *Potentilla erecta, Holcus lanatus* or *Nardus stricta*, as opposed to, for example, *Juncus articulatus* or *Equisetum fluviatile* (Fig. 3d). *Succisa pratensis* was situated centrally in ordination space.

Vascular plants
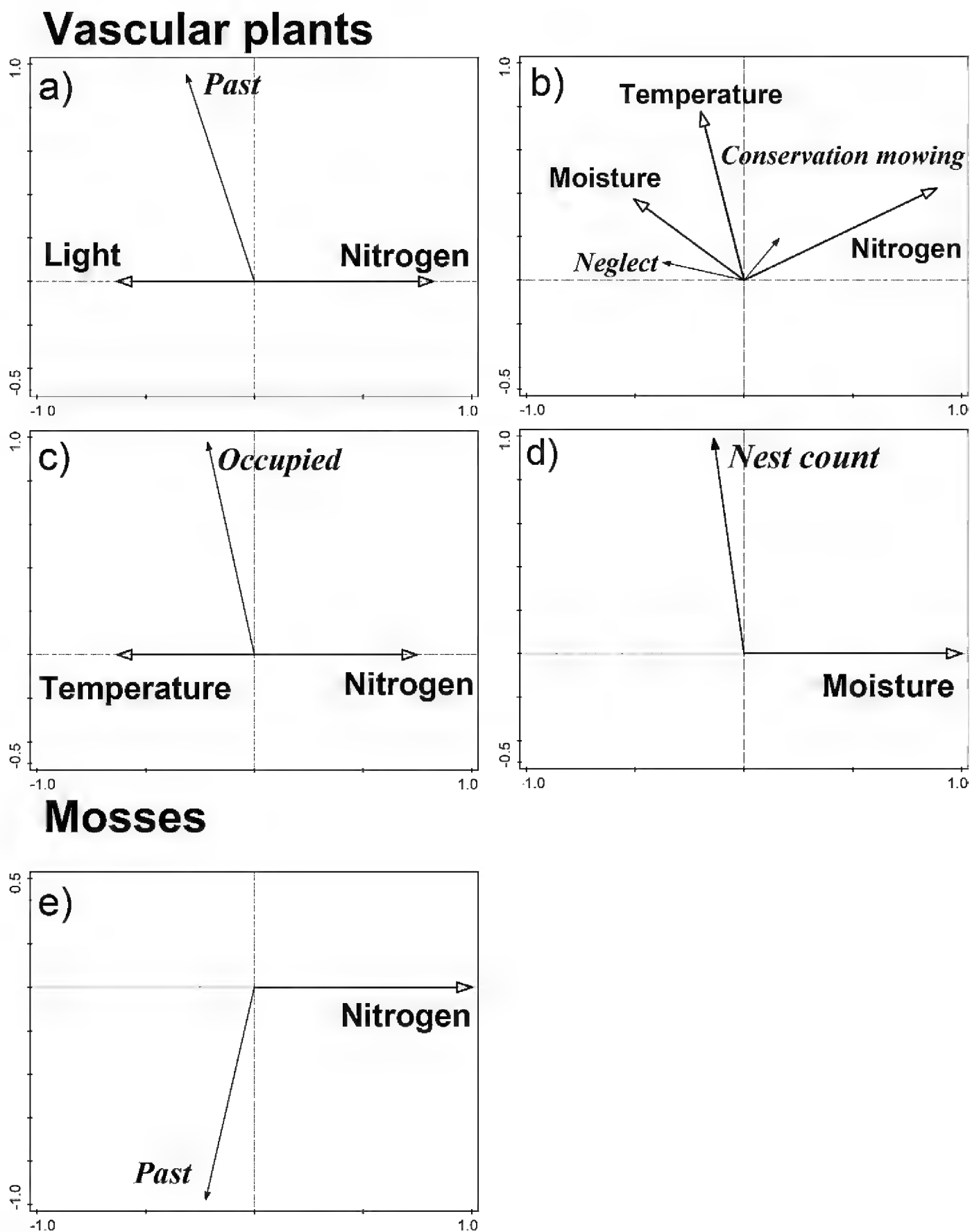
Mosses

Figure 3. Selection of RDA ordination biplots, relating the DCCA ordination axes (narrow full darts, with captions in italics) to Ellenberg's indicator values (empty darts, with captions in bold) of vascular plants' **a–d** and moss' **e** species, in the 66 relevés taken from *Euphydryas aurinia* sites. Individual panels illustrate ordination constrained by **a** survey **b** site management **c** *E. aurinia* occupancy **d** *E. aurinia* nests count **e** survey.

Tests of the specific hypotheses regarding interactions about predictors (Table 3) detected no significant relationship for mosses, but several relationships for vascular plants and all plants, implying that vascular plants drove the patterns for all plants. We reject the hypotheses (4a) that effects of 'management' on vegetation differed between the 'surveys', (4b) that 'occupancy' systematically changed between the 'surveys' and

Table 2. Results of DCCA ordinations testing the effects of individual predictors on plants species composition in the vegetation samples taken from localities of *Euphydryas aurinia*, including interpretations of the results by the constituent plant species' Ellenberg's indicator values. "n.a." stands for situations where no relationship to Ellenberg's values was found.

	Ax1	Ax2	Ax3	Ax4	Var%	F, P_{Ax1}	$F, P_{all\ axes}$	Interpretation by indicator values	Ax 1	Ax 2	Ax 3	Var%	F, P_{Ax1}	$F, P_{all\ axes}$
Vascular plants														
Geography[a]	0.083				3.6	5.8*		Ax1: −R −H −N	0.150			13.7	11.2***	
Site	0.102	0.031	0.023	0.009	10.6	0.6*	2.3*	+H +R +N	0.053	0.018	0.013	6.9	3.5***	5.8***
Survey	0.022				0.4	1.5**		+N −L (present)	0.093			8.3	9.8***	
Survey | Site	0.022				0.6	1.7***		+N −L (present)	0.097			8.7	10.3***	
Management	0.034	0.014			1.3	1.2**	1.9***	+H +L (neglect) +N (conservation mowing)	0.119	0.009		11.4	8.6***	9.3***
Management | Site	0.026	0.013			1.1	2.0*	1.6*	+H (neglect) +T +H (conservation mowing)	0.083	0.022		9.0	5.7***	7.4***
Occupancy	0.027				0.6	1.9**		+R +T (occupied)	0.073			6.3	7.5***	
Occupancy | Site	0.018				0.3	1.4***		−N +T (occupied)	0.046			3.8	4.6**	
Nests count	0.036				1.1	2.5**		+R −H (high count)	0.169			15.8	19.5***	
Nests count | Site	0.010				0.1	0.8**		−H (high count)	0.031			2.6	6.1*	
Mosses														
Geography[b]	0.395	0.034			6.6	10.1*	5.6*	+H	0.052			3.8	3.7*	
Site	0.449	0.072	0.019	0.009	10.0	10.7**	2.2+	+N	0.047			3.3	3.3*	
Survey	0.059				0.3	1.4***		+N (present)	0.049			3.5	3.8+	
Survey | site	0.056				0.6	1.8*		+N (present)	0.102			3.5	3.5+	
Management	0.144	0.012			1.8	3.5*	2.2*	n.a.						
Management | Site	0.108				1.5	2.9***	1.9**	n.a.						
Occupancy	0.054				0.2	1.3ns		−H +R (occupied)	0.103			7.6	7.7*	3.8*
Occupancy | Site	0.035				0.0	0.9ns		n.a.						
Nest count	0.104				1.2	2.5+		+R (high count)	0.046			3.2	3.3+	
Nest count | Site	0.018				0.0	0.5ns								
All plants														
Geography[a]	0.114				4.3	6.9*		−R	0.119			11.43	26.0***	
Site	0.135	0.034	0.023	0.013	10.5	0.6*	2.3*	+H −R	0.054	0.016		5.9	5.4**	7.1***
Survey	0.025				0.3	1.4*		+N −L (present)	0.095			8.6	10.1***	
Survey | Site	0.025				0.5	1.6***		+N −L (present)	0.099			9.0	10.5***	
Management	0.042	0.014			1.4	1.3*	1.9**	−N (neglect) −L +H (conservation mowing)	0.117	0.009		6.7	8.4***	9.2***
Management | Site	0.033	0.010			1.1	2.2*	1.7*	−N (neglect) −L +H +T (conservation mowing)	0.093	0.012		5.6	4.8***	5.5***
Occupancy	0.051				0.3	1.4**		+T +R (occupied)	0.066			5.6	6.8**	
Occupancy | Site	0.020				0.2	1.3**		+T −N (occupied)	0.047			3.7	4.7*	
Nest count	0.072				0.8	2.0**		+R −H (high count)	0.163			15.5	37.5***	19.4***
Nest count | Site	0.011				0.1	0.7*		−H (high count)	0.031			1.4	6.1*	

Legend: Ax1-Ax4 refer to ordination axes, Var% to variation explained by the respective ordination axis/model.
Geography covariate models structure: [a] ~latitude^2; [b] ~latitude +longitude +latitude^2
Monte-Carlo test results – ns: not significant, +: p < 0.1; *: p < 0.05; **: p < 0.01; ***: p < 0.001.

(4e) that 'management' influenced 'nest count'. The significant interaction 'survey*nest count' (4c) documented that nest numbers decreased at some occupied sites and increased at others, but these changes were not related to the plants' indicator values. The interaction 'management*occupancy' (4d) showed that conservation mowing of high moisture sites increased the chance of occupancy, while conservation-mowed dry sites tended to become unoccupied. This was apparent (Fig. 4) from association of such hygropilous plants as *Filipendula ulmaria*, *Scutellaria galericulata* or *Potentilla palustris* with the occupied conservation mown situations.

Discussion

Recent resampling of 66 vegetation relevés, taken from 12 sites occupied by the endangered butterfly *Euphydryas aurinia* in the Czech Republic 15 years ago and mostly managed for the benefit of the butterfly and its host plant during the interim period, revealed vegetation changes explicable by ecological requirements of the constituent plant species and demonstrably connected to the prosperity of *E. aurinia* colonies. The changes included both structural properties of vegetation, i.e. increased cover of trees, forbs and grasses and decreased cover of mosses and more subtle changes at the level of floristic composition.

The most evident change was the increased representation of plants tolerating high nitrogen, accompanied by decrease in plants preferring high values of light. This corresponded with the increase in tree layer and increased cover of both forbs and grasses. Increased nitrogen load due to increased atmospheric deposition (Nijssen et al. 2017) and runoffs from close environs (Stoate et al. 2001; Butler et al. 2008) is a commonly acknowledged problem, causing declines of poorly-competitive plants from grasslands across Europe (Bollens et al. 2001; Stevens et al. 2010; Payne et al. 2013), including the Czech Republic (Novotný et al. 2016). Plants utilising high nitrogen tend to be bulky and competitively dominant, outcompeting light-demanding plants by shading their habitats. It follows that *E. aurinia* sites in western Czech Republic are not spared the general threat of eutrophication reported for this species (e.g. Brunbjerg et al. (2017)) and its host plant (Vergeer et al. 2003; Holder et al. 2020) from other parts of Europe.

The increased indicator values for nitrogen correspond with decreasing cover of mosses. Although the mechanisms are still disputed, decreasing bryophytes representation and species richness from temperate grasslands are associated with increased nutrient levels and accompanying increase of grasses and forbs (Arróniz-Crespo et al. 2008; Duprè et al. 2010; Müller et al. 2012).

Nutrient loads of grasslands are amenable by management interventions, such as removal of the biomass by mowing (Pecháčková et al. 2010; Ziaja et al. 2017; Swacha et al. 2018; Yang et al. 2019; Sienkiewicz-Paderewska et al. 2020). In our results, however, the 'management' effect on plant species' composition was counterintuitive, with nitrogen-demanding plants inclining towards mowing (both conventional or conservation-minded) and relatively thermophilous plants inclining towards either conservation mowing or neglect. Before interpreting this paradox, we should admit
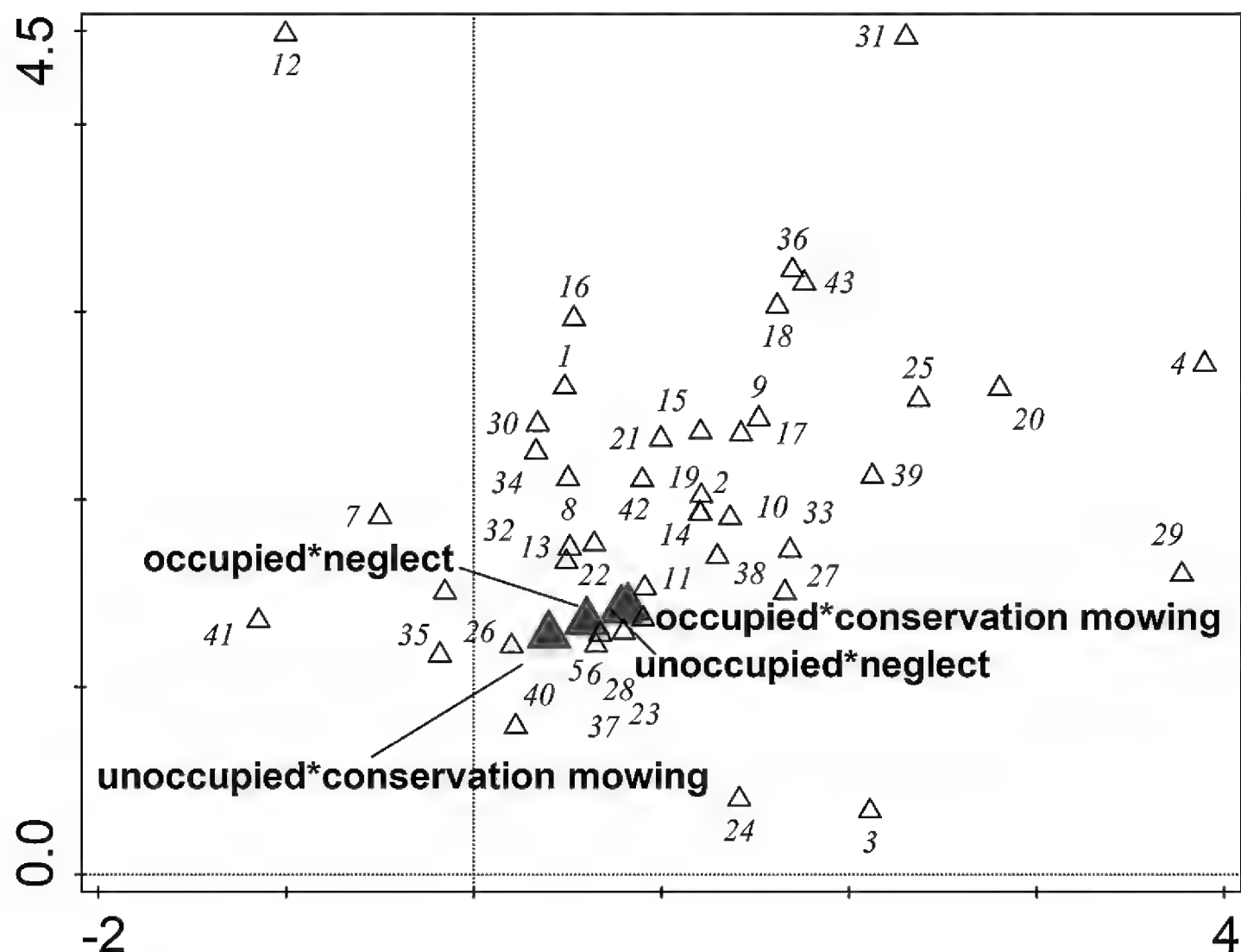
Figure 4. DCCA ordination biplot visualising the significant interaction between site management and occupancy of close environs of the vegetation relevés by larval nests of *Euphydryas aurinia*. Model for vascular plants: species composition ~ management * occupancy | management + occupancy + Site. See Table 3 for details. 1 – *Agrostis canina*, 2 – *Agrostis stolonifera*, 3 – *Achillea millefolium*, 4 – *Alnus glutinosa* juv., 5 – **Angelica sylvestris,** **6** – *Bistorta major*, 7 – *Caltha palustris*, 8 – *Cardamine pratensis*, 9 – *Carex echinata*, 10 – *Carex nigra*, 11 – *Carex panicea*, 12 – *Carex rostrata*, 13 – *Cirsium palustre*, 14 – *Crepis paludosa*, 15 – *Epilobium palustre*, 16 – *Equisetum fluviatile*, 17 – *Equisetum palustre*, 18 – *Eriophorum angustifolium*, 19 – *Festuca rubra*, 20 – *Filipendula ulmaria*, 21 – *Galium palustre*, 22 – *Galium uliginosum*, 23 – *Holcus lanatus*, 24 – *Hypericum maculatum*, 25 – *Juncus articulatus*, **26 – *Juncus conglomeratus***, 27 – *Juncus effusus*, 28 – *Lychnis flos-cuculi*, 29 – *Lysimachia vulgaris*, 30 – *Mentha arvensis*, 31 – *Menyanthes trifoliata*, 32 – *Myosotis nemorosa*, 33 – *Poa pratensis*, 34 – *Poa trivialis*, 35 – *Potentilla erecta*, 36 – *Potentilla palustris*, **37 – *Rumex acetosa***, 38 – *Sanguisorba officinalis*, 39 – *Scutellaria galericulata*, **40 – *Succisa pratensis***, 41 – *Tephroseris crispa*, 42 – *Valeriana dioica*, 43 – *Viola palustris*.

that our categorisation of interventions affecting the diverse sites over a decade and a half necessarily oversimplifies the matter. Whereas "neglect" is an unequivocal category, "conventional mowing" may vary amongst years in timing and numbers of cuts. The same applies for "conservation mowing", which is practised by concerned officers and volunteers, who adaptively react to the momentary situation at the sites (and *E. aurinia* populations), varying the timing, intensity and spatial extent of the interventions. Consequently, no intervention ("neglect") is more likely applied at nutrient-poor sites, such as dry heathlands or waterlogged bogs, whereas mowing intensifies in response to successional overgrowth. This probably generated the contradictory pattern for nitrogen and the expected pattern for thermal conditions. The absence of a significant effect

Table 3. Results of DCCA tests of specific hypotheses of interactions amongst predictors. In all models, significance of the interaction between variables a*b was tested by setting a, b and Site as covariables, y ~ a*b | a + b + Site. The resulting ordination model, if significant, was further interpreted by the constituent plants' Ellenberg's indicator values. "n.a." stands for situations when no relationship to Ellenberg's values was found.

Model (hypothesis)	Ax1	Ax2	Ax3	%Var	F, P_{Ax1}	$F, P_{all\ axes}$	Interpretation by indicator values
Vascular plants							
Survey*Management | Survey + Management + Site (**4a**)	0.015	0.015		0.2	0.6[ns]	1.1[ns]	
Survey*Occupancy | Survey + Occupancy + Site (**4b**)	0.013			0.0	1.0[ns]		
Survey*Nest count | Survey + Nest count + Site (**4c**)	0.008			0.1	0.6[*]		n.a
Management*Occupancy | Management + Occupancy + Site (**4d**)	0.019			0.4	1.5[*]		+H (eigenvalue 0.030, 2.5%, F = 6.0[*])
Management*Nest count | Management + Nest count + Site (**4e**)	0.013	0.001		0.0	1.0[ns]	0.8[ns]	
Mosses							
Survey*Management | Survey + Management + Site (**4a**)	0.082	0.013		0.5	2.2[+]	1.3[ns]	n.a.
Survey*Occupancy | Survey + Occupancy + Site (**4b**)	0.040			0.1	1.1[ns]		
Survey*Nest count | Survey + Nest count + Site (**4c**)	0.026			0.0	0.3[ns]		
Management*Occupancy | Management + Occupancy + Site (**4d**)	0.046			0.2	1.2[+]		n.a.
Management*Nest count | Management + Nest count + Site (**4e**)	0.052	0.020		0.0	1.4[ns]	1.0[ns]	
All plants							
Survey*Management | Survey + Management + Site (**4a**)	0.021	0.015		0.3	1.4[ns]	1.2[ns]	
Survey*Occupancy | Survey + Occupancy + Site (**4b**)	0.015			0.0	1.0[ns]		
Survey*Nest count | Survey + Nest count + Site (**4c**)	0.009			0.1	0.6[*]		n.a.
Management*Occupancy | Management + Occupancy + Site (**4d**)	0.022			0.4	1.5[*]		+H (eigenvalue 0.029, 2.9%, F = 5.8[*])
Management*Nest count | Management + Nest count + Site (**4e**)	0.017	0.008	0.003	0.0	0.4[ns]	0.8[ns]	

Legend: Ax1-Ax4 refer to ordination axes, Var% to variation explained by the respective ordination axis/model. Monte-Carlo test results – [ns]: not significant, [+]: p < 0.1; [*]: p < 0.05. \\192.168.83.6\docs1\Work\JOURNALS\NatureConservation\[WORK]\90452.

for the 'survey*management' interaction (specific hypothesis 4a) corroborated that the effects of management did not change between surveys in a systematic way.

Two predictors related to utilisation of the sites by the butterfly were 'occupancy' and 'nest count'. Although values of the predictors refer to broader areas than to specific relevés ('occupancy') and even to entire sites ('nest count'), our results point to the affinity of *E. aurinia* towards warmer and/or drier conditions with relatively high soil reaction. This agrees with findings from *E. aurinia* populations developing on *S. pratensis* elsewhere: a preference for sparser sward easily penetrated by light in Poland (Pielech et al. 2017); a unimodal response to moisture in Denmark (Brunbjerg et al. 2017); the preferred oviposition at plants surrounded by low sward in Wales (Pschera and Warren 2018); and occurrence of larval webs amidst intermediate sward height in Britain (Botham et al. 2011). Regarding populations utilising other host plants, Scherer and Fartmann (2022) reported a preference for relatively warmer microhabitats from *Scabiosa lucida* developing populations in hummocky meadows in the German pre-Alps. Although *E. aurinia* is often described as a moist grasslands/fens/bogs species, it arguably prefers drier and warmer patches amidst such grasslands. A common denominator of all these requirements appears to be low soil nutrients. In the mostly intensively farmed landscapes of temperate Europe, low-nutrients conditions were more likely preserved in remote landscapes and poorly-accessible localities.

It is, thus, tempting to view *E. aurinia* as a "refugee species", surviving in suboptimal areas or habitats due to human pressure on the optimal ones (cf. Kerley et al. (2012, 2020)).

Whereas all the above observations are correlational, the tests relating the interactions between the butterfly-related ('nest count', 'occupancy') and sites-related ('management', 'survey') predictors to the relevés' species composition (Table 3) directly assess the vegetation effects on *E. aurinia*. Amongst them, we view the significant 'occupancy*management' interaction as the most interesting result. An identical intervention type – in this case, conservation mowing exporting biomass and increasing thermal intake – supports *E. aurinia* at moister sites, but not at drier sites. Cases when site conditions modulate the impacts of conservation interventions might be rather common (e.g. Morris (2000), Helden et al. (2020), Dumont et al. (2020), Bussan (2022)) and if ignored, they may result in undesirable outcomes of well-intended activities. This highlights the necessity to flexibly adapt vegetation management to both local variation amongst sites and interannual variation in such aspects as rainfall or phenology. In our study system, within the Slavkovský les PLA, management of *E. aurinia* sites is administered by a single administrative unit, the practitioners have first-hand experience with the system and routinely adapt the interventions according to the momentary circumstances. More generally in the Czech Republic, however, "management plans" for nature reserves are issued for 10–15 years' duration and lack the necessary flexibility (Ministry of the Environment of the Czech Republic 2018). Still less flexible may be generic management prescriptions for non-protected lands, such as provisions for the EU agri-environmental payments. The lack of flexibility may explain why effects of such incentives are sometimes disappointing (Batary et al. 2011; Concepción et al. 2012; Merckx and Pereira 2015).

A more general problem with conserving *E. aurinia* sites in the Czech Republic stems from the fact that it targets only the pre-defined localities known to be occupied by the species at some time interval during the last two decades. Although so far successful – the butterfly is still there and most of the sites (except for a few at the margins of national distribution) are still occupied (Junker et al. 2021 and unpublished data), this approach does not cover all potentially habitable sites in the wider environs. Within the Slavkovský les PLO, management of the farmland "matrix" underwent substantial transformation during the last decades, from marginal and unprofitable arable fields, through seeding of species-poor grass mixture, to the current prevalence of beef ranching accompanied by hay production at large scales. Within this matrix, new patches with abundant presence of the *S. pratensis* host plant occasionally appear, calling for a more dynamic, landscape-orientated conservation strategy for *E. aurinia*, which would presumably be more robust with regard to recently changing moisture and temperature conditions.

Conclusions

A vegetation survey of sites inhabited by a butterfly of European conservation concern, *Euphydryas aurinia*, its replication after 15 years and interpretation of the vegetation changes using plants' indication values contributed to understanding both the

vegetation development and larval ecology of the butterfly. The vegetation changes observed may be characterised as increased nutrient load, causing shifts towards less heliophilous vegetation. This represents a potential problem for long-term existence of the butterfly, which prefers warmer, drier and less acidic conditions within otherwise cold, moist and base-poor sites for its larval development. Follow-up surveys that would document future vegetation development of the sites with respect to conservation interventions applied and changing climatic conditions are highly desirable. Even more fascinating results could be produced by periodic vegetation surveys of *E. aurinia* sites across the entire diversity of its habitats across Europe and beyond.

Acknowledgements

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Supplementary material 1

Ordination scores from the indirect DCA analysis species

Authors: Přemysl Tájek, Aleš Tenčík, Martin Konvička, Václav John

Data type: Plant community records

Explanation note: Excel sheet containing ordination scores from the indirect DCA analysis species ~ | site of vascular plants, mosses and all plants recorded in vegetation relevés taken from sites occupied by the *Euphydryas aurinia* butterfly.

Link: https://doi.org/10.3897/natureconservation.52.90452.suppl1